Mutation in codon 713 of the β amyloid precursor protein gene presenting with schizophrenia

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Following reports of mutations of codon 717 in exon 17 of the amyloid precursor protein (APP) gene in early-onset familial Alzheimer's disease, we screened exon 17 for new mutations in presenile dementia. The majority of the 105 patients screened had definite or probable Alzheimer's disease, but we also included atypical cases and some chronic schizophrenics. We identified a single abnormal case — a chronic schizophrenic with cognitive defects. Sequencing revealed a C to T nucleotide substitution which produces an alanine to valine change at codon 713. We were unable to detect the mutation in the remaining members of the original cohort nor in a further 100 chronic schizophrenics and 100 non-demented controls. Nonetheless, the position of the mutation in a critical portion of the APP gene suggests that it may well prove to be pathogenic.

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The β amyloid precursor protein (APP) gene located on chromosome 21 encodes a large protein which under certain, as yet poorly understood circumstances, is abnormally cleaved to produce the A4 peptide as one of its products. A4 has been isolated both from amyloid deposits in cerebral blood vessels and from senile plaques present in the brains of patients with Alzheimer's disease and older patients with Down's syndrome. Mutations of codons 693 and 717 have been found linked to illness in hereditary cerebral haemorrhage with amyloidosis of the Dutch type (HCHWAD) and in a small number of families (probably less than 1%) with early onset Alzheimer's disease, respectively[1-5]. Recently, a third mutation at codon 692 has been shown to cosegregate in one family with presenile dementia and cerebral haemorrhage[6]. Furthermore, transgenic mice overexpressing the A4 peptide coding region of the APP gene have some neuropathological features characteristic of Alzheimer's disease[7]. The APP gene appears therefore, to occupy a pivotal role in the molecular pathway that leads to Alzheimer's disease. The degree of phenotypic variation and/or clinical overlap caused by specific mutations at separate sites remains uncertain[8], as does the overall biological role of the APP gene itself.

In an attempt to answer some of these questions, we decided to screen for new mutations of the APP gene. We examined not only cases which fulfilled strict operational criteria for definite or probable presenile Alzheimer's

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                         *
                 689     692
-------tttgttttcaag GTG TTC TTT GCA GAA GAT GTG GGT TCA AAC AAA GGT
                 Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly
                             A→G

                                                 *       ↓       ***
                                                713              717
GCA ATC ATT GGA CTC ATG GTG GGC GGT GTT GTC ATA GCG ACA GTG ATC GTC
Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val Ile Val
                                                A->V             V->I
                                                                 V->F
                                                                 V->G

ATC ACC TTG GTG ATG CTG AAG AAG AAA CAG TAC ACA TCC ATT CAT CAT GGT
Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile His His Gly

GTG GTG GAG gtagg-------------
Val Val Glu
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Fig. 1 Analysis of exon 17 of the APP gene in proband III-1. *a*, Direct PCR sequence of exon 17 of the APP gene. The nucleotide substitution G to A resulting in A713V is indicated (C to T on the sense strand). *b*, Digestion of the 317 bp exon 17 PCR products with *MaeIII*. A713V creates a *MaeIII* restriction site and the heterozygous PCR product is cleaved to yield fragments of 187 bp and 130 bp. Normal DNA remains uncut.

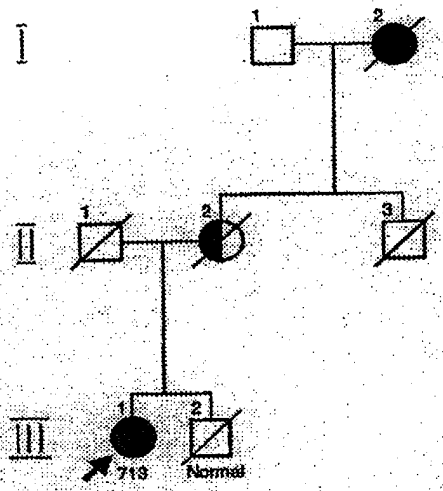

Fig. 2 Pedigree of proband. Shaded circle, schizophrenia; half shaded, deaf and blind in middle age; diagonal line, death. Arrow points to proband.

disease, but also a group with unusual/atypical presentations, including some schizophrenics whose illness had run a chronic and deteriorating course. A high proportion of schizophrenics have neuropathological abnormalities[9–11]. While no consistent pattern emerges, presumably due to the heterogeneous nature of the disorder, cerebral amyloid angiopathy[12] and early onset Alzheimer's disease[13] are both occasionally observed and families are reported that regularly present with schizophrenia a decade or so before classic symptoms of Alzheimer's disease predominate[13]. We hypothesized that disease of the APP gene would resemble that of the prion protein gene and be able to produce a variety of unexpected clinical subtypes[14].

APP mutation analysis

DNA from 105 patients was screened for mutations in exon 17 of the APP gene by the chemical cleavage mismatch technique. A mismatch was identified in one sample from a patient with chronic schizophrenia and cognitive defects. Subsequent direct screening of the relevant polymerase chain reaction (PCR) product revealed a C to T nucleotide change at base 1913 (numbered according to the APP770 transcript) at codon 713 (Fig. 1). This would substitute a valine for an alanine amino-acid residue and generates a new *Mae*III restriction site (Fig. 1).

The presence of this mutation was confirmed in DNA from a second blood sample taken from the patient, but was not found in the remaining 104 patients with Alzheimer's disease, a further 100 unrelated patients with chronic schizophrenia, nor in 100 non-demented controls (data not shown).

Proband background

The proband, III.1 (Fig. 2), is a 62 year old female with over thirty years of chronic schizophrenia. In spite of neuroleptic medication she has suffered recurrent episodes of acute illness with florid thought disorder, paranoid delusions and auditory hallucinations necessitating thirteen separate admissions to psychiatric hospital. She was also at one time given fortnightly maintenance electroconvulsive therapy. When not acutely ill she has been variously described as apathetic, lacking in drive, forgetful and requiring coaxing to perform simple household chores. The overall clinical picture of the proband can be described as a schizophrenic defect state with evidence of progressive cognitive decline — in other words, early superimposed Alzheimer's disease cannot be ruled out. Over the last eighteen months the proband has been reported as more forgetful, which was the original reason for her inclusion in the mutation screening. However this problem has proved fluctuant, and recent neuropsychological testing with the Weschler Adult Intelligence Scale (WAIS) gave a verbal intelligence quotient of 107, performance 97 and overall score 103, that is, I.Q. within normal limits. Her memory quotient is 100, but she has poor performance on logical memory, associate learning and current information. Electroencephalography performed twice within 8 months displays frequent runs of generalized diffuse slow theta and delta waves maximal in the frontal and temporal regions. Computer tomography scanning of the brain shows cerebral atrophy with markedly widened sulci in both frontal and temporal lobes with dilatation of the lateral and third ventricles. This is confirmed on MRI scanning which also shows bilateral focal cortical loss and multiple high intensity signal lesions scattered throughout the white matter (data not shown). In conclusion, organic brain disease is present whose neuroradiological appearance is compatible with but not diagnostic of an ischaemic type of dementing illness.

Family history

The proband is the older of two children (see Fig. 2). Her brother, III.2, who died of melanoma two years ago, had a subarachnoid haemorrhage aged 57 from an anterior communicating artery aneurysm. Angiography and computer tomography at the time of the bleed showed no other cerebral abnormalities. DNA was extracted from paraffin embedded blocks of an axillary resection for melanoma performed shortly before death. Sequencing of the PCR amplified product revealed a normal exon 17. The mother of the proband (II.2) had no documented psychiatric history but by age 62, had become blind and deaf — cause unknown. She is then reported to have had a cerebral thrombosis before dying aged 67 of a myocardial infarction. The maternal grandmother, on whom detailed case records were available, was a psychiatric inpatient for over thirty years with schizophrenia phenotypically indistinguishable from that of the proband. She was also reported as forgetful. She died suddenly aged 65. Several other members on the maternal grandmother's side of the family are also reported as insane. In spite of an extensive search we have not yet been able to trace any living affected cases to examine for the APP mutation. By contrast systematic enquiry revealed no psychiatric or neurological illness on the paternal side of the family, and no members on either side had typical early onset Alzheimer's disease.

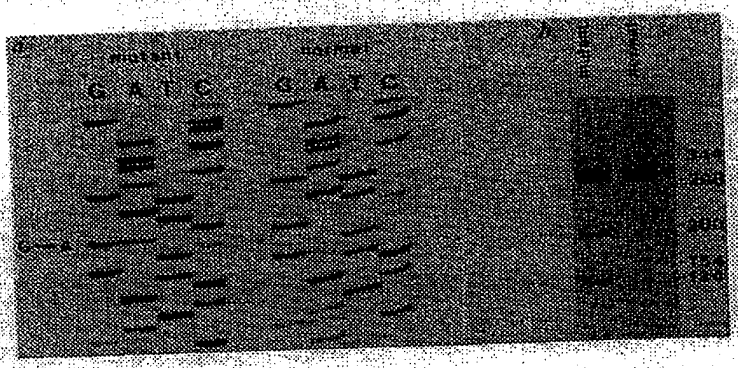

Fig. 3 DNA and amino acid sequence of exon 17 of the APP gene which encodes the C-terminal portion of the A4 peptide. The mutation described in this article occurs at codon 713 and would substitute a valine for an alanine residue. The arrow indicates the C-terminus of the longest A4 peptide reported (43 residues) although cleavage does also occur at the neighbouring residue (713) yielding a 42 residue peptide. The position of the mutation causing Hereditary cerebral hemorrhage with amyloidosis of the Dutch type (HCHWA-D) at amino-acid residue 693(ref. 1) is indicated, as in an Ala to Gly substitution at position 692 which cosegregates in a family with presenile dementia and cerebral haemorrhage (ref. 6). Three mutations in early-onset Alzheimer disease at codon 717 have also been described.

Discussion

Lack of linkage data and neuropathology as well as mutation analysis restricted to two members of the family means that interpretation of our findings must be cautious. It is entirely possible that the mutation in codon 713 is a rare non-pathogenic variant of no medical significance, which by chance we have initially encountered in the family described. Alternatively, like mutations in codon 717, it may be pathogenic and be responsible for some or all of the neuropsychiatric problems described in the family. Several pieces of evidence argue against the former interpretation. Mutation 713 occurs in an expressed part of the APP gene which is highly conserved across species (Fig. 3). Such mutations are rarely non-pathogenic. Nor has it been found on screening many hundreds of control samples worldwide. Moreover, it is present in a crucial region of exon 17 flanked on one side by codon 717 (whose principal mutation also involves a C to T transition and is likely to have occurred by the same mechanism of deamination of a methylated cytosine situated 5' to guanine), and on the other side by the transmembrane A4 peptide cleavage site currently localized to codons 711–714(refs 15,16).

In contrast to mutations of codon 717, the mutation described here at codon 713 does not seem to produce Alzheimer's disease. In spite of extensive clinical and genealogical investigations of the family we found no cases with typical early-onset Alzheimer's features. Rather, the principal lesions of the brain, as the neuroradiology indicates, are focal and either ischaemic or haemorrhagic. We cannot be certain whether they are responsible for schizophrenic symptoms as well as the cognitive deficits observed. Nevertheless, given the variety of phenotypes associated with mutations of codons 692, 693 and 717, it is reasonable to suppose that mutation 713 may also be pleiotropic enough in its effects to produce an equally wide range of neuropsychiatric symptoms.

These effects may result from direct damage to specific brain regions as indicated from vascular amyloidosis, and/or Alzheimer type changes in the parenchyma. Alternatively, more subtle effects may arise from disturbance of the homeostatic role that the APP gene appears to play in maintaining the integrity of nerve cell function. Neuronal stress causes increased APP transcription[17], and when severe for example, following head injury, A4 peptide deposition[18]. It is also possible that mutation 713, situated as it is at the A4 peptide

Table 1 Clinical and demographic data on subjects examined for mutation codon 713

	Presenile Dementia n=105	Schizophrenia n=100	Non demented controls n=100
Diagnostic features	Alzheimer neuropathology (n=30)	DSMIIIR criteria for schizophrenia and RDC criteria for chronic schizophrenia (n=100)	No Alzheimers on neuropathology (n=53)
	Probable Alzheimers (NINCDS criteria) (n=58)		Other non-demented clinical referrals (n=47)
	Possible Alzheimers/Atypical cases (n=17)		
Mean Age of Onset (±sd)	57±6 yr	Before 45 yr	78±6 yr[a]
Range	28–64 yr (onset)	15–44 yr	67–91 yr[a]
Familial	1st Degree Relative demented n=20	1st or 2nd Degree Relative Schizophrenic n=24	–
Male/female	37/58	67/33	45/55

a, age at death of subjects with Neuropathology.

Acknowledgements

We thank Morag Jennie and her colleagues who helped in the clinical work-up of the family and the MRC Unit Registry staff — Alison Fordyce and Rhona le Mey for detailed genealogical analysis. I. Best supervised and interpreted MRI scanning. Judith Young prepared many of the DNA samples. We also thank Elaine Perry and Alec Gordon neuropathological assessments. Myfanway Thomas, M. Rana, D. Blackwood and W. Muir also provided patient blood samples and H. John Evans helped with the manuscript. This work was in part support by the Ludovici Bequest to the University of Edinburgh, Medical Research Council and Scottish Rite Schizophrenia Research Programme. D.St.C. is a Wellcome Trust Senior Research Fellow in Clinical Science.

cleavage site, may alter fine balances, such as by preventing one or more proteolytic enzymes cleaving the A4 peptide[19,20] and in certain circumstances, in a way analogous perhaps to mutation 178 of the prion protein gene causing fatal familial insomnia[21], present in early adult life with schizophrenia. These questions need answering, not least to determine whether blocking A4 peptide cleavage can be used as a pharmacological option in Alzheimer's disease. Further clinical investigations should resolve current uncertainty concerning the phenotype associated with mutation APP 713. It will also be important to characterize and compare transgenic animals with mutations at residues 713 and 717 described in humans. Until then the clinical implications of our findings must remain unclear.

Methodology

Patients. DNA was prepared from 105 cases who met NINCDS (National Institute of Neurological & Communicative Disorders and Stroke) criteria[22] for definite (n=30), probable (n=58) or possible atypical (n=17) presenile dementia Alzheimer's type (Table 1). The latter category also included two patients who fulfilled RDC (Research Diagnostic Criteria (St Louis))[23] for chronic schizophrenia. Average age of onset was 57±6 yrs (range 28–64 yr); there were 20 familial cases with a first degree relative affected. All definite cases had neuropathological confirmation of Alzheimer's disease (C.M.Y. and E. Perry). Others were recruited through psychiatric colleagues in Scotland with special request for familial cases. All were assessed by a consultant psychiatrist. DNA was also available on 100 non demented controls, 53 neuropathologically validated — average age 78 ± 6 yr (C.M.Y.), the others routine referrals to the genetics laboratory. A further 100 subjects who met RDC criteria for chronic schizophrenia were later recruited — see below, mostly from long stay psychiatric wards throughout Scotland.

PCR amplification. DNA extracted from peripheral blood leucocytes or post-mortem brain tissue was PCR amplified for exon 17 of the APP gene using the following conditions: 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 250ng DNA in a total volume of 100 µl. Amplification was performed after an initial cycle of 94 °C for 2 min 30 s, 55 °C for 30 s and 72 °C for 1 min followed by 91 °C for 30 s, 55 °C for 30 s then 72 °C for 1 min followed by 91 °C for 30 s, 55 °C for 30 s then 72 °C for 1 min for 30 cycles and a final 10 min extension time at 72 °C. The primer sequences used were 5'-CCTCATCCAAATGTCCCCGTCATT and 5'-GCCTAATTCTCTCATAGTCTTAATTCCCAC.

Mutation detection. The chemical cleavage mismatch method[24] was used to screen exon amplified 17 of the APP gene for mutations and was performed essentially as described[25]. DNA (courtesy of I. Hardy) from a patient with the APP 717 Val to Ile mutation served as a positive control for the method. An abnormal cleavage product other than that seen for the 717 mutant control DNA was detected in the DNA from one patient. Subsequent direct PCR sequencing demonstrated a C to T substitution at codon 713 in exon 17 (Fig. 1a). 1µg of Geneclean purified PCR product was sequenced using a Pharmacia T7 sequencing kit following the kit protocol, except that 80 picomoles of primer was annealed to the DNA by heating to 100 °C for 2 min and then plunged directly into ice. Dimethyl sulphoxide (1µl) was added to each termination mix to discourage reannealing of the dissociated DNA strands.

Screening for the codon 713 mutation. The C to T nucleotide substitution at codon 713 creates a *MaeIII* restriction site. Amplification of exon 17 gives a 317 bp product. In mutant DNA, *MaeIII* digestion gives fragments of 187 bp and 130 bp, while normal DNA remains uncut (Fig. 1b). Dot blot hybridization using labelled oligonucleotides specific for the normal or mutant 713 sequence (5'-GTCATAGCGACAGTGATC and 5'-GATCACTGTCACTATGAC respectively) was performed as described method previously[26]. The hybridization and wash temperatures for the normal and mutant oligos were 55 °C and 53 °C respectively.

Received 21 April; accepted 20 May 1992.

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